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Studies on preservation of sugarcane juice using hurdle technology

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Abstract
Hurdle technology is an emerging concept in which a particular hurdle or an obstacle is provided to the food product in order to prevent or slow down the growth of enzymes by ensuring its quality and safety. One such hurdle used in here is pasteurization treatment at high temperature and for less holding time. Pasteurization treatment at high temperature and for less holding time was found to be an effective method to reduce the microbial load and PPO activity and hence preserve the color and flavor of sugarcane juice. A study was conducted to observe the effect of pasteurization treatment at different temperature (80, 90, 100 °C) and holding time (60, 90, 120 sec) to enhance the shelf life of juice. Among different tests, it was observed that pasteurization treatment at 90 °C for holding time 90 sec was found to produce the best quality with reference to different quality attributes. In general, the combination was selected as it showed maximum decrease in reducing sugar, PPO activity and higher sensory scores. Therefore pasteurization at 90 °C for 90sec found to be an optimum treatment for preservation of sugarcane juice.

Keywords: Sugarcane juice, hurdle technology, pasteurization treatment, color, TSS, reducing sugar, polyphenol oxidase activity, microbial load, storage

Introduction
Sugarcane (*Saccharum offcinarum*) is a cash crop belongs to the family of grass Poaceae. In India it is cultivated mainly for sugar and juice (direct consumption). It is our pride that India became the largest sugar producing country in 2018-19 beating out Brazil for the first time in 16 years and produced about 33 million metric tonne of sugar. Cane juice is one of the energy booster, full of carbohydrates and iron, medically juice is useful for the treatment of enlarged prostate, reduce acidity, ailments like cystites, nephritis and gonorrhea. As it is alkaline in nature and due to the high concentration of magnesium, iron, potassium, and calcium, it prevents cancer. Its regular use can help to gain weight to under nutrition person (Kalpana *et al.,* 2013) [4]. Sugarcane juice after extraction cannot be stored on more than 24 hours with its original taste and aroma even in a chilled condition that is the reason why we see expellers everywhere all the year. The problem associated with it is after extraction it get spoiled due to the fast fermentation as it contains about 0.5% reducing sugar, 15-18% sucrose, mineral salts and adequate amount of organic nitrogen for microbial growth (Qudsieh *et al*., 2002) [8]. pH more than 4.6 makes it favourable for growth of pathogens. Major factor contributing quality loss in food and beverages is enzymatic browning which changes the color of the beverage due to presence of polyphenol oxidase enzyme (PPO). Contamination of juice is also the main reason of its spoilage, due to the environment in which it is prepared resulting rapid damage to taste, color and flavor. Hurdle Technology happens to be a new and efficient concept and technique for improving the quality of food and to enhance its shelf life. Basically the hurdle is a kind of barrier or an obstacle provided to a food product in order to prevent or slow down the growth of enzymes. The implementation of any kind of hurdle is to be done in such a way that it does affect the quality and originality of the food. Once the factors responsible to damage the sugarcane juice are defeated or killed permanently, evolution world wild in cold drinks sector would be achieved.

Materials and Methods

Preparation of Sugarcane Juice

The study was conducted at "College of Agricultural Engineering and Technology" and the raw materials were procured from the farm field itself.

The sugarcanes were peeled by eliminating the joints to reduce the microbial load which can be present at surface and joints.

Thereafter canes and extractor both were cleaned with KMS (potassium metabisulphite) with water before extraction of juice.

Then fresh sugarcane juice was extracted and was filtered by using muslin fabric cloth.

Application of Pasteurization Treatment

The oven was used to pasteurize the fresh cane juice extracted after standardization to pH 4.6 and TSS 14 ºBrix. It was pasteurized at temperature 80, 90, 100°C for 60, 90, 120 seconds. After the treatment given the samples were taken out cooled and filtered as sediments were deposited on the top of the juice. The treated juice was thereafter filled in sterilized glass bottles.

Fig 1: Peeled Sugarcanes

Fig 2: Pasteurized sugarcane juice

Table 1: Study parameters

Independent variables	Dependent variables
Fixed parameters	After extraction, juice was standardized to a certain TSS, pH i.e. TSS (14 ^{0} Brix), pH (4.6)
Pasteurisation- Temperature (℃) – 80, 90, 100 Holding time(sec.) – 60, 90, 120	Colour TSS Titrable acidity Microbial load Total phenolic content Reducing sugar PPO inactivation Sensory quality

Color measurement

The color measurements were performed using a digital colorimeter (CR-20 Konica Minolta, Tokyo, Japan) which shows the values as L^*, a^*, b^*. Total color change (ΔE) was obtained to know the difference in color of treated sample from control and was calculated by given equation.

$$\Delta E = \sqrt{(L_2-L_1)^2 + (a_2-a_1)^2 + (b_2-b_1)^2}$$

Total soluble solids (TSS)

The TSS of the sugarcane juice was measured by refractometer which determines the total soluble solids and is expressed in degrees Brix.

Titrable acidity

Titrable acidity was calculated using sodium hydroxide as titrant. It is calculated by the formula:

$$Titrable\ acidity(\%) = \frac{Equivalent\ wt.of\ acid\ \ X\ vol.of\ NaOH\ required\ X\ \ 0.1}{10X\ wt.of\ sample\ taken}$$

Total Phenolic Content

The total phenolic content was found by using Folin-Ciocalteu reagent method. In a testube 0.5 ml of sample was taken and leveled up by addition of 7.5 ml distilled water. Thereafter 1 ml of 50% Folin-Ciocalteu reagent was added and after 5 min, 1 ml (20%) of Na_2CO_3 was added. The absorbance reading was taken after 45 min at room temperature at 720 nm using spectrophotometer.

$$Total\ phenolic\ content\ (mg\ GAE/100ml) = \frac{Concentration\ of\ sample\ after\ absorbance\ reading}{wt.of\ sample\ taken\ x\ 10\ x\ 0.5}$$

Reducing Sugar

The reducing sugar was determined by using DNS (3, 5-dinitrosalicyclic acid) solution in which about 0.1 ml of the sample of juice was equalized with 5.9 ml of distilled water in testube. 3 ml DNS was added in the sample and the test tubes were heated in boiling water for 5 min. Then test tubes were taken out and while they were warm, 1 ml of Rochelle salt

solution was added in it. It was cooled and the absorbance reading was taken at 510 nm.

$$Reducing\ sugar(\%) = \frac{Concentration\ x\ 1}{50\ x\ volume\ of\ sample\ taken}$$

Polyphenol oxidase (PPO) activity

PPO activity was calculated by using 0.2 M catechol solution and 50 M phosphate buffer (pH 6.5). In a test tube, a mixture was prepared by addition of 4 ml of phosphate buffer and 2 ml of catechol solution and 1 ml of sugarcane juice was added. The absorbance reading was carried out at every 1 min interval at 420 nm. One unit of PPO activity was defined as 0.001DA420/min (Ozoglu & Bayindirli, 2002) [7]. Sample's activity was measured in terms of % residual PPO Activity (RA) as given in the equation.

Unit of ppo/ml = (Abs_f – Abs_i) / 0.001* time

Microbial Load

To determine the microbial load media was prepared by suspending 13 mg of nutrient broth in 1000 ml of water with 2% agar powder of the solution for bacteria test. Sterilized media was autoclaved at 121°C (15 lbs pressure) for 15 minutes. The media was the properly mixed before dispensing into the sterilized petriplates. The petriplates are kept in laminar air flow chambers for 20-25 min under the surveillance of UV light to solidify them. In sterile saline solution of 0.85g/100 ml, the pasteurized juice samples were diluted and were spread in petriplates. The plates were incubated at proper temperature and time i.e at 37°C for 24 h to obtain bacterial colonies. Thereafter the bacterial colonies were counted in which there were colonies between 30-300. Colonies formed per unit was calculated by formula:

$$CFU/ml = \frac{Number\ of\ colonies\ x\ dilution\ factor}{Volume\ of\ sample\ plated}$$

Sensory Evaluation

Sensory evaluation was done by 10 panelists through 9-point hedonic rating test method. The color, flavour, taste and overall acceptability were analyzed by the panelist of control and the treated samples.

Statistical Analysis

The statistical analysis was carried out to establish significant difference among the samples. All the experimental data was analyzed by analysis of variance (ANOVA) using MS EXCEL 2007 at 5% (P< 0.05) significance level

Result and Discussions

The results are obtained of control and treated samples and quality parameters of fresh (control) sugarcane juice are tabulated in the below table:

Table 2: Quality parameters of Fresh sugarcane juice

Quality parameters	Control sample
Color parameters	L^*= 24.3, a^* =4.4, b^*=27.3
pH	5.5
Total soluble solids (°Brix)	19.5±0.03
Titrable acidity (%)	0.258±0.03
Total phenolic content (mg of G.A.E /100ml)	55.85±1.61
Reducing sugar (%)	5.05±0.17
PPO activity (unit of ppo /ml of juice)	33.03± 1.14
Microbial load (log cfu/ml)	7.31±1.08
Sensory evaluation	9±0

Sugarcane juice was further standardized to TSS of 14° Brix by diluting the sugarcane juice and pH was adjusted to 4.6 by adding citric acid.

Most preservative attempts to prolong the shelf life of sugarcane juice have focused on pasteurization treatment by adjusting to a certain pH and TSS. (Bhupinder *et al*., 1991; Yusof *et al*., 2000) [10]. The effect of pasteurization treatment on quality parameters of sugarcane juice is shown in the table.

Table 3: Effect on quality parameters of sugarcane juice after pasteurization treatment

Treatment & time (sec.)	Color change (ΔE)	TSS (°brix)	Titrable acidity (%)	Total phenolic (mg of G.A.E /100ml)	Reducing sugar (%)	PPO (unit of ppo /ml of juice)	Microbial load, log(cfu/ml)	Sensory evaluation
80°C, 60s	18.36±0.2	14±0	0.21±0.07	37.76±1.01	3.99±0.08	1.25±0.25	4.9±0.18	8.33± 0.28
80°C, 90s	17.27±0.26	14±0	0.18± 0.02	33.84±0.34	3.08±0.12	1.25±0.66	4.7±0.18	8.33± 0.28
80°C, 120s	15.31± 0.4	14±0	0.16± 0.05	30.71±0.47	3.05±0.22	1.23±0.44	4.6±0.3	8.16± 0.28
90°C, 60s	16.57±0.18	14±0	0.16±0.143	27.80±0.71	2.95±0.14	1.08±0.38	3.9±0.9	8.16± 0.28
90°C, 90s	15.6± 0.19	14±0	0.14±0.002	26.99±1.59	2.54±0.11	0.91±0.52	3.5±0.18	8.16± 0.28
90°C, 120s	15.4± 0.47	14±0	0.13± 0.01	22.76±1.11	2.48±0.29	0.88±0.53	3.3±1.1	8±0
100°C, 60s	14.3±0.03	14±0	0.13± 0.02	19.87±0.71	2.14±0.13	0.83±0.44	2.9±1.2	7.66± 0.28
100°C, 90s	14.1± 0.4	14±0	0.12± 0.006	17.42±1.59	1.95±0.07	0.60±0.09	2.8±0.8	7.16±0.28
100°C, 120s	13.7± 0.36	14±0	0.11± 0.01	16.21±1.11	1.70±0.14	0.55±0.09	2.7±0.9	7±0
CD (5%)	2.5	-	0.036	2.85	0.38	0.31	4.07	0.25

Color measurement

The color parameters L^* a^* and b^* value changed with increase in temperature and time. All the values gradually increased as temperature increased with time. This might be due to the inactivation of enzymes at higher temperature which leads to increase in L^* value.

Sugarcane juice treated with 80°C for 60s showed highest change in color i.e. 28.2±0.22 and 100°C for 120s showed lowest color change i.e. 18.64±0.33. Therefore, at higher temperature and with longer holding time ΔE value decreased. Similar study was done by Azhari *et al*. (2018) [1]. ΔE decreased significantly as temperature increased and but with effect of time, there was no significant difference observed.

Total Soluble Solids

There was no significant change in TSS for all the temperature and time combinations as earlier the TSS was adjusted to 14°Brix. Huang *et al*. (2015) [3] found similar findings where thermal pasteurization does not affect the TSS of sugarcane juice.

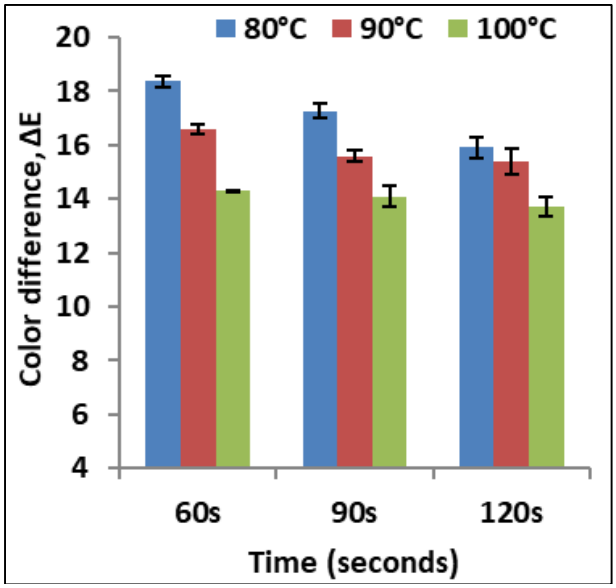

Fig 3: Effect of pasteurization treatment on color change

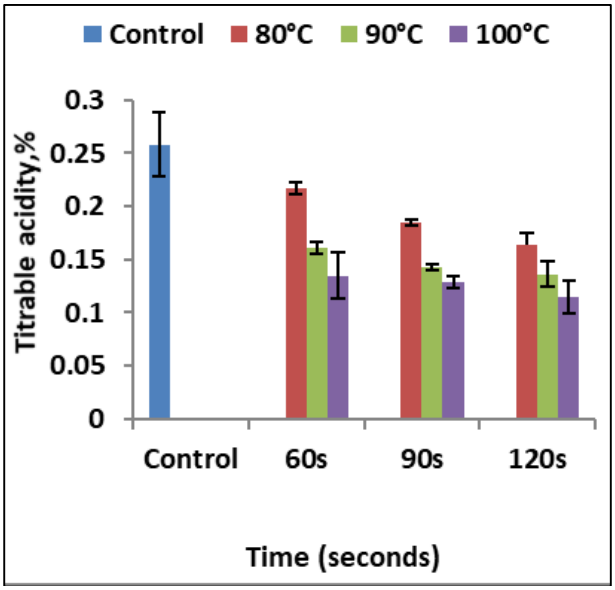

Fig 5: Effect of pasteurization treatment on titrable acidity

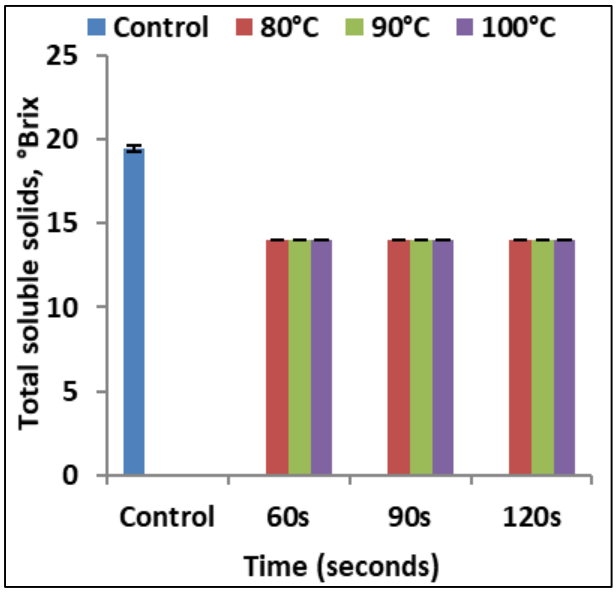

Fig 4: Effect of pasteurization treatment on TSS

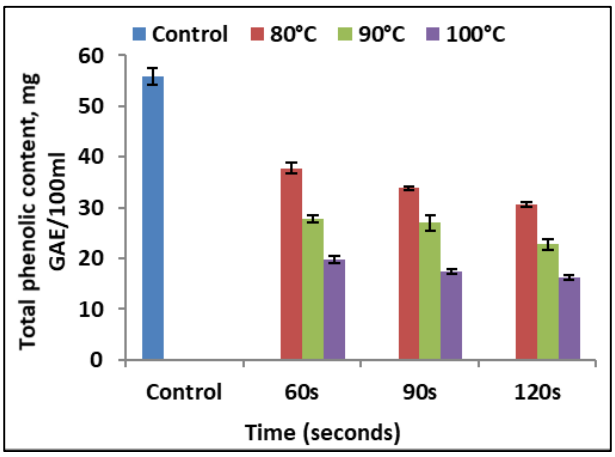

Fig 6: Effect of pasteurization treatment on total phenolic content

Titrable acidity

The acidity of the samples decreased with increase in temperature and time. It was observed that decrease in acidity for higher temperature and for longer time was more which could be due to the inactivation of enzymes at higher temperature treatment.

Similar study was done by Huang *et al.* (2015) [3] where acidity decreased but not significant due to thermal treatment. There was no significant difference in acidity. However, with effect of time, acidity decreased significantly. Therefore, time had significant effect on acidity but no significant effect of temperature was there.

Total phenolic content

The phenolic content decreased significantly with treatment. The highest value was observed for the sample treated with 80 °C, for 60s where it decreased to 32.39% whereas lowest value was observed at 100 °C for 120s where it decreased to 70.97% with respect to control sample.

With both effect of temperature and time phenolic content decreased significantly.

Reducing Sugar

Reducing sugar decreased to 21% at 80°C for 60s which was higher than the sample treated with 100°C for 120s which showed lowest reducing sugar i.e. decreased to 66.33% as compared to control sample. It was observed that at higher temperature for longer holding time reducing sugar decreases, as sucrose content in sugarcane juice changed due to the effect of pasteurization treatment. There was significant decrease in reducing sugar with increase in temperature. However, there was no significant decrease in reducing sugar when time increased with temperature. Similar study was done by Begum *et al.* (2015)

PPO activity

The main objective of the study is to prevent the browning of the sugarcane juice which is caused by the polyphenol oxidase enzyme. The PPO activity of control was about thirty folds higher than treated samples. The PPO activity decreased to 96.21% at 80°C for and at 100°C for 120s it decreased to 98.33% with respect to control sample. PPO can be inactivated at high temperature thermal treatment. However, a slight degreening was observed at higher treatment temperature, which suggests there was chlorophyll degradation of sugarcanes at higher temperature which led to the degreening. There was significant decrease in PPO

activity with increase in temperature, however with effect of time there was no significant decrease in PPO activity. Similar findings were made by Vamos-Vigyazo *et al.* (1981) [9], in which it was studied that PPO can be inactivated at high temperature thermal treatment

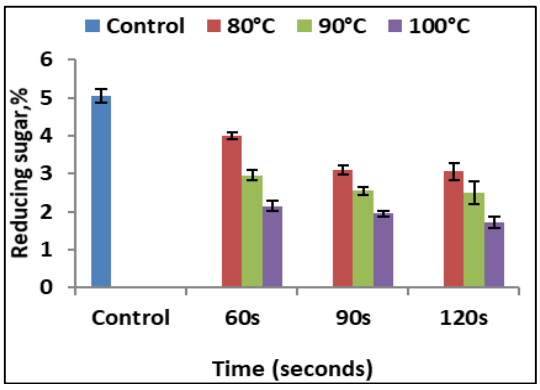

Fig 7: Effect of pasteurization treatment on reducing sugar

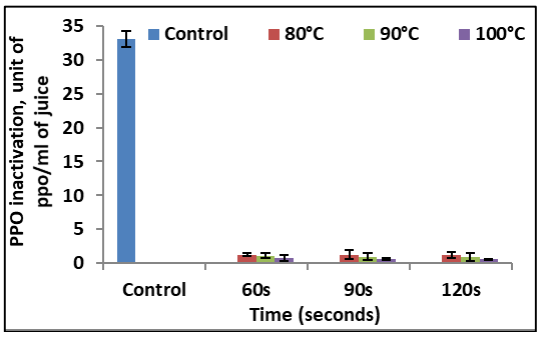

Fig 8: Effect of pasteurization treatment on PPO activity

Microbial load

The pasteurization treatment of sugarcane juice achieved a 3 log reduction in microbial count. Treatment at 80°C for 60s led to decrease in microbial load to 33% and treatment at 100°C for 120s led to 63% with respect to control sample. Similar results were obtained by Chauhan *et al.* (2002) [2] on bacterial count of sugarcane juice. There was significant difference in microbial load with effect of temperature as well as with effect of time.

Sensory evaluation

There was significant decrease in sensory scores with effect of temperature but no significant decrease was seen with time. It was observed that as treatment temperature and time increased the sensory scores decreased. Similar findings were made by Kunitake *et al.* (2014) [6].

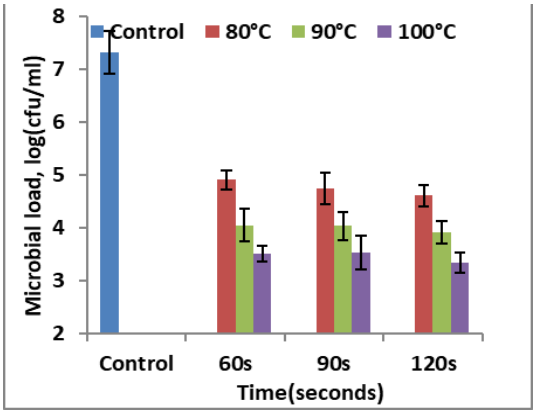

Fig 9: Effect of pasteurization treatment on microbial load

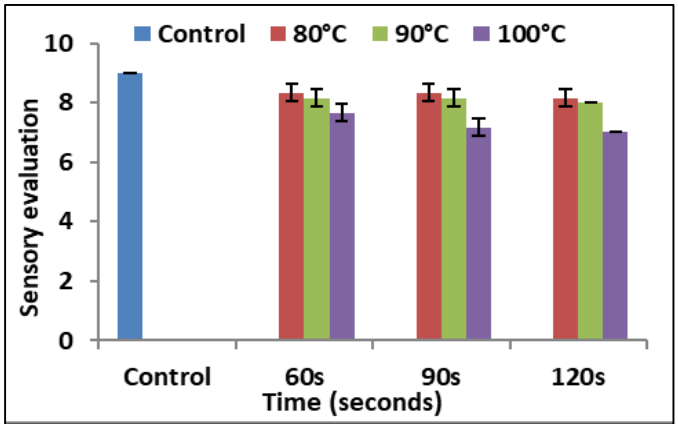

Fig 10: Effect of pasteurization treatment on sensory evaluation

Optimization of Quality Parameters of Sugarcane Juice

The optimization was done according to the changes in quality parameters after the treatment. The analysis was done to obtain the best desirable outputs i.e. minimum change in color, TSS, phenolic content and sensory evaluation and maximum decrease in reducing sugar, polyphenol oxidase activity and microbial load. The most desirable output obtained for color, TSS, titrable acidity, total phenolic content, reducing sugar, polyphenol oxidase activity, microbial load and sensory evaluation were as 15.6, 14°Brix, 0.143%, 27 mg GAE/100ml, 2.54%, 0.91unit of ppo/ml of juice, 3.5 log cfu/ml and 8.66 respectively corresponding to the pasteurization treatment at 90°C for 90 seconds. In general, the combination was selected as it showed higher decrease in reducing sugar, PPO activity and higher sensory scores.

Conclusion

The pasteurization treatment after the standardization of juice used as hurdle in treatment of the cane juice. Therefore, treatment of sugarcane juice at 90°C for 90sec holding time was found to maintain the quality of juice with higher sensory scores, maximum decrease in PPO activity, reducing sugar and microbial load. Further the treated juice can be stored to increase the shelf life.

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